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### Research on molecular relationship between fatty acid metabolism and selective lignin degradation in *Ceriporiopsis subvermispora*

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*Ceriporiopsis subvermispora*, a white-rot fungus, is characterized as one of the best bio-pulping fungi because it can selectively degrade lignin without serious damage to cellulose. We have demonstrated that the same fungus produces large amounts of unsaturated fatty acids (UFAs), such as linoleic acid (18:2n-6), and degrades lignin by manganese peroxidase-catalyzed lipid peroxidation (LPO). In spite that UFAs might act as precursors of LPO, however, the molecular relationship between UFA biosynthesis and lignin degradation in white-rot fungi including *C. subvermispora* has remained to be elucidated; therefore, we focused on UFAs, linoleic acid in particular, which is a potential precursor of LPO involved in the selective lignin degradation by *C. subvermispora*.

Firstly, we have cloned a cDNA fragment containing one open reading frame (ORF) using the PCR-based methods. This ORF product had similarities with various fungal  $\Delta 12$ -fatty acid desaturases, which converts oleic acid (18:1n-9) to linoleic acid. The same product had three histidine cluster motifs (His-box), which are known to be the consensus sequence of fatty acid desaturases. Using the SOSUI system to classify and predict the secondary structure of membrane proteins, moreover, this ORF product was estimated to be a membrane-bound protein with four transmembrane helices. These observations strongly suggested that this ORF encodes a  $\Delta 12$ -fatty acid desaturase in *C. subvermispora*. We designated this ORF as *Cs-fad2*.

For heterologous expression of *Cs-fad2*, we constructed an expression plasmid in which *Cs-fad2* cDNA was oriented to a constitutive promoter of the alcohol dehydrogenase 1 gene (*ADH1*). After transformation of the expression plasmid in budding *Saccharomyces cerevisiae* lacking a  $\Delta 12$ -fatty acid desaturase gene, we performed fatty acid analysis of the yeast transformant carrying *Cs-fad2*. A single peak in the chromatogram of gas chromatography analysis was detected specifically for fatty acid methyl esters from the transformant and its retention time was identical to that of authentic methyl linoleate (C18:2). Gas chromatography-mass spectrometry analysis of the fatty acid methyl derivative demonstrated that its mass peak was at  $m/z$  294, the same molecular mass methyl linoleate, and that the fragmentation pattern was identical to that of authentic standard of methyl linoleate. These results indicated that the *Cs-fad2* gene encodes for *C. subvermispora*  $\Delta 12$ -fatty acid desaturase, which converts oleic acid to linoleic acid.

We also performed transcriptional analysis of *Cs-fad2* in various culture conditions. The transcription of *Cs-fad2* was activated and repressed in the presence of a lignin fragment like vanillin and exogenous fatty acids, respectively. Because vanillin is a key intermediate found during lignin biodegradation, the transcriptional activation of *Cs-fad2* by vanillin is very intriguing, and also seems to support our previous results that *C. subvermispora* produces large amounts of linoleic acid at an early stage of wood decay. These results may shed light on the molecular relationship between fatty acid metabolism and selective lignin degradation in *C. subvermispora*.

#### Reference

[1] Watanabe, T., Tsuda, S., Nishimura, H., Honda, Y., and Watanabe, T. (2010) Characterization of a  $\Delta 12$  fatty acid desaturase gene from *Ceriporiopsis subvermispora*, a selective lignin-degrading fungus. *Appl. Microbiol. Biotechnol.* 87: 215-224.